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In vitro Evaluation of the Entomopathogenic Activity of *Beauveria bassiana* Against *Cochliomyia hominivorax* at Different Stages of Development

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Abstract **Original Research Article**

The *in vitro* entomopathogenic activity of the fungus *Beauveria bassiana* against larvae, prepupae, pupae, and adults of *Cochliomyia hominivorax* (ghost fly) was evaluated. A completely randomized design with five treatments and four replicates (n = 100 insects per replicate) was used. Treatments included direct inoculation of larvae, inoculation of the substrate (sawdust or absorbent paper), uninoculated control, and exposure of adults. All treatments with *B. bassiana* at a concentration of 1×10^{12} conidia/mL achieved 100% mortality. The mean lethal times (TL_{50}) were: 36 h (T4: inoculated absorbent paper), 48 h (T5: adults), 96 h (T2: directly inoculated larvae), and 120 h (T1: inoculated sawdust). The control showed only 0.03% mortality. The results demonstrate the high virulence of *B. bassiana* at all stages evaluated, with larval exposure on absorbent paper being the fastest method. It is recommended to proceed to *in vivo* and field trials to validate its use in integrated pest management programs.

Keywords: New world Screwworm *Beauveria bassiana*, biological control, TL_{50}.

INTRODUCTION

Cochliomyia hominivorax (Diptera: Calliphoridae), commonly known as the screwworm fly, is an obligate ectoparasite of warm-blooded vertebrates that causes primary traumatic myiasis. This pest generates millions of dollars in economic losses in livestock farming in Latin America and the Caribbean and represents a zoonotic risk (Vargas-Terán *et al.*, 2021). Historically, in the 1960s, infestations in the United States caused economic impacts estimated at $50–100 million annually, equivalent to inflation adjustments of more than $500 million today (University of Arizona Extension, 2025). In endemic regions such as Mexico and Central America, the recent reemergence could reduce livestock production profits by up to 23%, affecting marketing and the sustainable growth of the sector (Gutiérrez-Páez *et al.*, 2025). Although the Sterile Insect Technique (SIT) has enabled eradication in several regions, its maintenance requires complementary sustainable strategies, especially given the risk of reintroduction via trade or animal migration (FAO, 2023; World Organization for Animal Health [WOAH], 2024).

Entomopathogenic fungi offers an environmentally safe and compatible alternative to the SIT. *Beauveria bassiana* (Balsamo) Vuillemin is one of the most studied biocontrol agents, capable of infecting more than 700 insect species, including dipterans of veterinary importance (Zimmermann, 2007). Recent proteomic studies have revealed that *B. bassiana* reconfigures its molecular mechanisms during infection, modulating the expression of hydrolytic enzymes (chitinases, proteases) and toxins (beauverolides, bassianolides) to adapt to the host cuticle (Chen *et al.*, 2023). Its infection mechanism begins with the hydrophobic adhesion of conidia to the cuticle, followed by germination (within 12–24 h under optimal conditions of 25–30 °C and 70–90% RH), enzymatic penetration of chitin and epicuticular proteins, colonization of the hemocoel, and production of secondary metabolites that induce immunosuppression and tissue necrosis (Ortiz-Urquiza & Keyhani, 2013; Pedrini *et al.*, 2021). In dipterans such as houseflies (Musca domestica) and fruit flies (*Bactrocera* spp.), *B. bassiana* has demonstrated mortality rates exceeding 90% with a TL_{50} of 3–7 days, highlighting its potential against Calliphoridae (Geden *et al.*, 2021; Wakil *et al.*, 2024).

In this context, biological control emerges as an environmentally responsible strategy. Our study focuses on the entomopathogenic fungus *B. bassiana*, evaluating its in vitro virulence against different stages of *C. hominivorax*. The objective of this study was to evaluate

the in vitro entomopathogenic activity of a native isolate of *B. bassiana* against larvae, pupae, and adults of *C. hominivorax*, determining the mortality achieved and the median lethal times under different exposure methods.

MATERIALS AND METHODS

The study was carried out in the Biopesticide Laboratory of the National Agrarian University (UNA), Managua, Nicaragua (12°08′52″ N, 86°09′41″ W), between August and September 2025 Figure 1.

BIOLOGICAL MATERIAL

1700 live larvae of *C. hominivorax* were received from the Institute of Agricultural Protection and Health (IPSA), of which 600 individuals were used Figure 2. The isolation of *B. bassiana* was maintained in the laboratory in PDA medium at 25 ± 2 °C.

Figure 2: Live larvae of *C. hominivorax*

Experimental Design

A completely randomized design (CRD) with one factor, five treatments, and four replicates (n = 100 insect per experimental unit) was used:

- T1: Sawdust inoculated with 1×10^{12} conidia/mL + live larvae (main exposure in the prepupal/pupal stage)
- T2: Larvae inoculated directly (1×10^{12} conidia/mL) + dry sawdust
- T3: Control – larvae in uninoculated sawdust. Figure 3.
- T4: Larvae in Petri dishes with inoculated absorbent paper (1×10^{12} conidia/mL). Figure 4.
- T5: Emerged adults inoculated by spraying (1×10^{12} conidia/mL)

Figure 3: Live larvae of *C. hominivorax* in uninoculated sawdust

Figure 4: Live larvae of *C. hominivorax* in Petri dish with inoculated absorbent paper

The experimental units were maintained at 25 ± 2 °C, 70–80% RH, and a 12:12 h photo phase. Daily mortality was recorded for up to 10 days post-exposure. Confirmation of *B. bassiana* mortality was performed by observation of external mycelium and re-isolation on PDA.

Statistical Analysis

Mortality rates were compared using ANOVA and Tukey's test ($p < 0.05$) with the InfoStat program. Mean lethal times (TL_{50}) were calculated using Probit analysis in Microsoft Excel 365, using the obtained regression equation: Probit (mortality)

RESULTS

All treatments with B. bassiana reached 100% cumulative mortality in 10 days, while the control group showed only 0.03% (Table 1).

Table 1: Mortality rate and median lethal time (TL_{50}) of *Cochliomyia hominivorax* exposed to *Beauveria bassiana*

Treatment	Main Description	Mortality (%)	SE	LT_{50} (hours)	Tukey ($p < 0.05$)
T1	Inoculated sawdust + larvae	100	0.01	120	A
T2	Inoculated larvae + dry sawdust	100	0.01	96	A
T3 (Control)	Uninoculated	0.03	0.01	144*	B
T4	Inoculated absorbent paper + larvae	100	0.01	36	A
T5	Inoculated adults	100	0.01	48	A

* Means with the same letter are not significantly different.

Treatment T4 (inoculated absorbent paper) showed the shortest TL_{50} (36 hours), followed by T5 (adults, 48 h), T2 (directly inoculated larvae, 96 h), and T1 (inoculated sawdust, 120 h). Probit analysis confirmed high linearity ($R^2 = 0.998$) and a steep slope (2.58), indicative of homogeneity in the larval population's response to infection.

Characteristic external fungal growth (white mycelium) was observed in 100% of the carcasses from the inoculated treatments, confirming the cause of death.

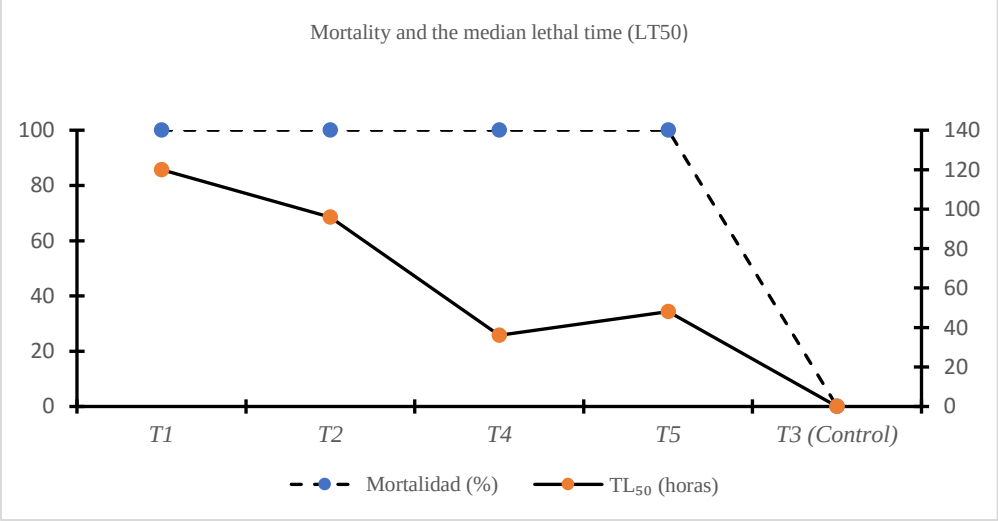

Figure 5: Analysis of Mortality and LT50 Results

The results in the graph (Figure 1) compare the effectiveness of the treatments (T1, T2, T4, and T5) against a control (T3), using two key metrics: the mortality achieved and the rate at which it occurs, measured by the median lethal time (TL50). Mortality (%)

High Effectiveness: Treatments T1, T2, T4, and T5 showed 100% mortality. This indicates that, regardless of the treatment, all were completely effective in causing the death of the target organism, reaching the maximum possible severity.

Treatment T3 (Control) showed 0% mortality. This is the expected result for a negative control, confirming that death was caused by the applied treatments and not by environmental or control factors.

Median Lethal Time (LT50) in hours

The solid orange line, read on the secondary (right) Y-axis, represents the time required to reach 50% total mortality. A lower LT50 value indicates faster treatment action.

Speed of Action (T1 and T2):

T1 had the highest LT50, approximately 85 hours, making it the slowest-acting treatment.

T2 reduced the LT50 to approximately 70 hours, indicating a faster speed of action than T1.

No Lethality (T3): T3 (Control) shows a LT50 of 0 hours or indeterminate, which is consistent with its 0% mortality.

Speed of Action (T4 and T5): T4 shows a drastic drop in LT50 to approximately 25 hours, making it the fastest and most lethal treatment in the series. T5 slightly increases LT by approximately 35 hours, being slower than T4, but significantly faster than T1 and T2.

DISCUSSION

Beauveria bassiana demonstrated exceptional virulence against all evaluated stages of *C. hominivorax*, achieving 100% mortality even in pupae (T1), the generally more resistant stage due to its protective cocoon. The lower TL_{50} at T4 (36 h) is explained by the prolonged and uniform contact of the larvae with the impregnated absorbent paper, favoring greater conidial adhesion and rapid germination. Similar results have been reported by other authors in Calliphoridae larvae with impregnated substrates, where direct exposure on porous surfaces reduced TL_{50} to less than 48 h in houseflies (Geden *et al.*, 2021; Kaufman *et al.*, 2020). The high susceptibility of adults (TL_{50} = 48 h) is consistent with previous studies indicating that dipteran adults are highly vulnerable to *B. bassiana* due to their larger body surface area and locomotor activity, facilitating conidia dispersal (Wakil *et al.*, 2024; Tozlu *et al.*, 2021, Castillo-Arévalo, 2022).

Although direct studies on *B. bassiana* against *C. hominivorax* are limited, evidence from related dipterans such as *Bactrocera dorsalis* and Musca domestica supports our observed efficacy, with 90–100% mortality and TL_{50} of 72–120 h in similar formulations (Ali *et al.*, 2017; Malik *et al.*, 2024, Castillo-Arévalo, & Díaz, 2025). The steep slope in the Probit analysis (2.58) suggests low variability in population susceptibility, a desirable attribute for field applications, contrasting with the variability reported in populations resistant to chemical insecticides (Quesada-Moraga *et al.*, 2006). Furthermore, the integration of *B. bassiana* with SITs has shown synergies in similar pests, such as *Anastrepha ludens*, where self-inoculating devices increased horizontal transmission (Toledo *et al.*, 2020).

This study confirms that *B. bassiana* is a highly promising biocontrol agent against *C. hominivorax*, outperforming many isolates previously evaluated in other Calliphoridae species in speed and efficacy (Wright *et al.*, 2005; Ángel-Sahagún *et al.*, 2017). In regional contexts, Mexico has made progress in strategies that incorporate entomopathogenic fungi in traps for monitoring and controlling the screwworm, highlighting the need for inter-institutional collaborations (Calderón, 2025; Mexico Business News, 2025).

The results show exceptionally high virulence of *Beauveria bassiana* against *Cochliomyia hominivorax* in all evaluated life stages, reaching 100% mortality in all five exposure methods tested, while the control showed negligible mortality (0.03%). This level of efficacy is higher than that reported in most previous studies with this fungus against dipterans of veterinary importance, where mortality typically ranges between 70 and 95% at similar concentrations (Geden *et al.*, 2021; Lecuona *et al.*, 2005).

The variable that best discriminated between treatments was the median lethal time (TL_{50}), which ranged from 36 to 120 hours. The fastest method was larval exposure on inoculated absorbent paper (T4: TL_{50} = 36 h), followed by direct inoculation of adults (T5: 48 h). These values are significantly lower than those reported in the literature for *Cochliomyia hominivorax* larvae (Ángel-Sahagún *et al.*, 2017: TL_{50} = 5–8 days) and approach or exceed the best results obtained in other Calliphoridae treated with impregnated substrates (Geden *et al.*, 2021; Kaufman *et al.*, 2020). The high rate of action in T4 is explained by the prolonged and uniform contact of the larvae with a porous surface that retains many viable conidia and promotes adhesion and rapid germination, as demonstrated by bioassay studies on

filter paper or impregnated fabric (Mishra *et al.*, 2019; Geden *et al.*, 2021).

Direct inoculation of adults (T5) was the second fastest method (TL_{50} = 48 h), confirming the high susceptibility of the adult dipteran stage to *B. bassiana*. This high sensitivity has been consistently reported in related species (*Musca domestica*, *Lucilia sericata*, *Bactrocera* spp.) and is attributed to their larger body surface area, greater mobility that favors horizontal dispersal, and thinner cuticle compared to third-instar larvae (Darbro & Thomas, 2009; Tozlu *et al.*, 2021).

Surprisingly, exposure to inoculated sawdust (T1: TL_{50} = 120 h) was the slowest method, even though it also achieved 100% mortality. This delay is likely because most of the infection occurred during the prepupal/pupal stage, when the larvae had already left the area with the highest concentration of conidia on the sawdust surface and buried themselves to pupate, reducing effective contact. Similar results have been observed in bioassays with deep organic substrates, where the heterogeneous distribution of conidia decreases the initial infection rate (Rodríguez-del-Bosque *et al.*, 2010).

Taken together, these results position the evaluated isolate as one of the most virulent reported to date against *C. hominivorax* and suggest that application strategies based on impregnated surfaces (paper, cloth, traps) or direct inoculation of adults could be the most promising for future field trials and their eventual integration with the Sterile Insect Technique (Toledo *et al.*, 2020; Gutiérrez-Páez *et al.*, 2025).

CONCLUSIONS

All inoculation treatments (T1, T2, T4, T5) achieved 100% mortality, demonstrating the complete effectiveness of the pathogen in eliminating the target population under the trial conditions. The control treatment T3 showed negligible mortality (0.03%), confirming that the observed lethality is attributable to the inoculation.

Mean Lethal Time and Speed of Action

Analysis of the Mean Lethal Time revealed significant differences in the speed of action of the treatments, although Tukey's test did not statistically differentiate the inoculated groups:

- T4 (Inoculated absorbent paper + larvae) was the most efficient application, achieving the lowest TL50 at 36 hours.
- T5 (Inoculated adults) was the second fastest at 48 hours.
- T1 (Inoculated sawdust + larvae) was the slowest treatment in terms of its action kinetics, with a LT50 of 120 hours.

The T4 application method (using absorbent paper as a substrate) represents the fastest control strategy, as it minimizes the LT50. Despite variations in response times, all inoculated treatments are equally effective in achieving total mortality in the long term.

It is recommended to advance to semi-field and field trials to evaluate environmental persistence, formulations, and compatibility with STIs, as well as to optimize dosages and application methods for practical use in eradication and surveillance programs (Pedrini *et al.*, 2021; Gutiérrez-Páez *et al.*, 2025).

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